IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of: Docket No.: 06510003PB

Marshall Schwartz

Serial No.: 09/931,112 Group Art Unit: 1631

Filed: August 17, 2001 Examiner: M. BORIN

For: **TREATMENT OF INTESTINAL EPITHEIAL CELL MALFUNCTION, INFLAMMATION OR DAMAGE WITH HEPATOCYTE GROWTH FACTOR**

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

1. I am the inventor of the above-identified patent application, Serial No. 09/931,112.

2. I am a Professor of Surgery and Pediatrics at Thomas Jefferson University. I also am a Professor of Surgery of Pediatrics at Drexel University School of Medicine and St. Christopher's Hospital for Children.

3. Inflammatory bowel disease (IBD) is an inflammatory condition that may occur anywhere in the gastrointestinal tract, including the stomach, the small intestine, which may be further subdivided into the duodenum, jejunum and ileum, and the large intestine, which may be subdivided into the cecum, ascending, transverse, descending and sigmoid colon and rectum. Ulcerative colitis and Crohn's disease are distinct disease processes that are collectively referred to as IBD. Additionally, severe acute gastroenteritis, chronic gastroenteritis, cholera, chronic infections of the bowel, immunologic disorders affecting the intestine, and immunodeficiency syndromes affecting the intestine, are additional inflammatory disease processes of the bowel. Necrotizing enterocolitis (NEC)

is also an inflammatory condition of the bowel primarily affecting infants. Although the etiology of IBD and the other inflammatory disease processes of the bowel are still unknown, the pathogenesis is thought to involve immunological factors, environmental influences and genetic susceptibility.

4. Hepatocyte growth factor (HGF) is a pleiotropic growth factor with activity in the central nervous system, the lung, the kidney, and in the intestine, in addition to the liver. HGF has been shown to enhance epithelial cell proliferation in both the lung and intestine (I also demonstrated that HGF enhanced mucosal growth and intestinal absorption *in vivo*). Furthermore, HGF levels have been found to be elevated in humans following both peritonitis and IBD.

5. The purpose of my research studies, which formed the basis for this Patent Application, was to determine if HGF could be demonstrated to have potential clinical use in patients afflicted with IBD. These series of studies were specifically designed to determine if HGF would positively influence the inflammatory process in a patient afflicted with IBD.

6. Transfection of the HLA-B27 gene into normal Fisher rats induces a spontaneous chronic inflammation of the gastrointestinal tract which is similar to the lesions seen in patients with IBD. Therefore, using this rat model of IBD, my research studies demonstrated that administration of HGF decreased ulceration and hemorrhage appreciated on gross analysis of the bowel as shown in Exhibit 1. Exhibit 1 shows gross pictures of the bowel from samples taken from the cecum (top), the jejunum (middle) and the ileum (bottom) from normal Fisher rats (F344) (left panel), untreated HLA-B27 rats (middle panel), and HGF-treated HLA-B27 rats (right panel). As shown in Exhibit 1, the normal Fisher rats demonstrated no ulceration; the untreated HLA-B27 rats had large areas of ulceration and hemorrhage (dark red stain); and the HGF-treated HLA-B27 rats showed reduced ulceration and hemorrhage compared to the untreated HLA-B27 rats. Furthermore, when gross mucosal damage of the bowel in untreated HLA-B27 rats and HGF-treated HLA-B27 rats was quantitatively assessed, the mucosal damage in HGF-

treated HLA-B27 rats was statistically significantly reduced compared to untreated HLA-B27 rats (Exhibit 2).

7. Furthermore, this research demonstrated that administration of HGF decreased inflammation of the bowel appreciated in the histological pictures attached as Exhibit 3. In Exhibit 3, normal Fisher rats showed normal intestinal architecture (the intestinal villi staining as blue) and did not show any signs of inflammation, edema or hemorrhage; the untreated HLA-B27 rats showed signs of significant edema (white areas), hemorrhage (red stained areas), and inflammation as demonstrated by infiltration of white blood cells; and the HGF-treated HLA-B27 rats demonstrated an improved intestinal architecture (blue stain) with a significant reduction in edema, hemorrhage and inflammation compared to the untreated HLA-B27 rats. Moreover, when histological lesions were scored by a "blinded" independent observer using a previously established histologic lesion index in the normal Fisher rats, untreated HLA-B27 rats and HGF-treated HLA-B27 rats, a statistically significant reduction in lesion score was demonstrated in the HGF-treated HLA-B27 rats. *See* Exhibit 4.

8. Moreover, an additional study performed at my direction evaluated the potential anti-inflammatory role of HGF as a basis for the reduction in bowel inflammation rather than its role as a growth factor. Here, I have demonstrated the unexpected results that HGF treatment of HLA-B27 rats resulted in a statistically significant decrease in protein expression of two inflammatory cytokines, tumor necrosis factor-α (TNF- α) and interferon-γ (IFN- γ) in HGF-treated HLA-B27 rats compared to untreated HLA-B27 rats. *See* Exhibits 5 and 6 respectively. Furthermore, semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) was also performed which demonstrated that HGF decreased the RNA expression of TNF- α and IFN- γ. These unexpected findings clearly demonstrate that HGF has a direct or indirect effect on these inflammatory mediators. Thus, these findings demonstrate that the mechanism of action for the clearly demonstrated benefit of HGF treatment in this model of IBD is its anti-inflammatory capability by blocking the effects on the mucosa of inflammatory

mediators rather than accelerating the proliferation of intestinal mucosa and replace the damaged and inflamed intestine.

9. All statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and such willful false statements may jeopardize the validity of the application or any patents issuing thereon.

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Date 11/29/05